Available online at www.sciencedirect.com

ScienceDirect

Procedia Food Science 3 (2015) 96 – 103

Procedia Food Science

The First International Symposium on Food and Agro-biodiversity (ISFA2014)

The Potency of Endophytic Fungal Isolates Collected from Local Aromatic Rice as Indole Acetic Acid (IAA) Producer

Syamsia[a], Tutik Kuswinanti[b], Elkawakib Syam'un[b] and Andi Masniawati[c]

a. Agriculture Faculty of Muhammadiyah University, Makassar
b. Agriculture Faculty of Hasanuddin University, Makassar
c. Dept. of Biology, Faculty of Natural Science, Hasanuddin University, Makassar

Abstract

Endophytic fungi are fungi that live in plant tissues without causing disease symptoms and abnormalities in plants. This study aims to obtain endophytic fungal isolates from local aromatic rice and screening of their ability to produce the IAA hormone as a plant growth promoter. Totally 16 endophytic fungal isolates were isolated from aromatic rice tissue of Pulu Mandoti. Ten isolates obtained from stem, three isolates from the root and three isolates from leaf tissue. The ability of endophytic fungal isolates in producing hormones IAA varied from 0.635 to 2.651 mgl^{-1}. Similarly, the ability to dissolve phosphate also varied from 0.005 – 3.719 mgl^{-1}, and there is only 1 isolates that caused abnormal growth in rice seedlings.

Peer-review under responsibility of the organizing committee of Indonesian Food Technologist Community

Key words: endophytic fungi, local aromatic rice, IAA

INTRODUCTION

South Sulawesi is one of the aromatic rice producing areas in Indonesia. Pulu Mandoti has higher level of fragnant than other aromatic rice in this area, and cultivated only by people in the District Salukanang Enrekang.

* Corresponding author.
E-mail address: syamsiatayibe@yahoo.co.id

Peer-review under responsibility of the organizing committee of Indonesian Food Technologist Community
doi:10.1016/j.profoo.2015.01.009

Endophytic fungi are microorganisms that presence in plant tissues or organs such as seeds, leaves, flowers, twigs, stems and roots. Various functional compounds can be produced by endophytic fungi, and can act as anti cancer, antiviral, antibacterial, antifungal, plant growth hormones, insecticides and others [1.2]

Indole acetic acid is essential compound for the growth and development of roots and shoots, many microbes including Plant Growth Promoting Rhizobacteria (PGPR) produce IAA [1]. Soil fungi and endophytes secrete plant growth-promoting substances such as indole acetic acid (IAA) and gibberellins [3.4.5]

Auxin was first isolated and characterized as plant growth, and indole-3-acetic acid (IAA) is a type of auxin [6.7]. Most of the genus Trichoderma produce auxin indole acetic acid (IAA), with or without precursor L-tryptophan [8]. Trichoderma isolated from rhizospher were more efficient in producing IAA compared *T. asperellum* T211 [9]. Deshwal *et al*. [10.11], observed that IAA produced by *Pseudomonas aeruginosa* MR-9 increases plant height, dry weight, number of nodules per plant, nodule fresh weight of *Mucuna pruriens* as many as 184, 124, 139, 180% compared with controls.

Essentially Phosphorus in the soil is presence in unavailable form for plants, and organisms associated with the plants can help in dissolving the mineral P to facilitate absorption by plants. The fungus has been reported to dissolve P by producing of organic acids and is known to have a higher efficiency than bacteria in dissolving of phosphorus [12]

The main objective of this research is to obtain endophytic isolates from local aromatic rice that has the ability to produce IAA hormones and dissolved phosphorus.

MATERIALS AND METHODS

Isolation of Endophytic Fungus

Local aromatic rice plant samples used in this study is Pulu Mandoti which is aromatic rice that has the most fragrant aroma and the highest economic value among 8 types of aromatic rice that is cultivated by farmers in this regions. Samples taken from rice plantation located in District Salukanang. Isolation of endophytic fungi carried out on the roots, stems and leaves of the local aromatic rice plants. Sterilization plant parts done gradually by soaking them for 60 seconds in 70% ethanol, 3% NaOCl for 60 seconds, and 70% ethanol for 30 seconds. Then rinsed four times with sterile distilled water dried on sterile filter paper. Cut a small part of the plant to be grown in PDA medium.

Extract preparations Endophytic Fungus

Endophytic fungi isolates that have been sucultured on PDA (Potato Dextrose Agar) and incubated for 7 days. Five disks of fungal colony put on to liquid medium Potato Dextrose Broth (PDB) were incubated at 28^{o}C in a shaker with 150 rpm/min for 7 days, and centrifuged at 5000 rpm for 25 min. The supernatan transferred in to new flask and the pellet was removed .

Detection and Quantification of IAA

Capability of endophytic fungi in producing IAA hormones was done using the method used by Bhagobathi and Joshi (2009)[13] . Supernatan of endophytic fungi mixed with Salkowsky reagent then incubated for 20 minutes. Observation of the color change before absorbance was measured using a spectrophotometer with a wavelength of 535 nm. IAA concentration of each isolate was compared to a standard curve.

Phosphate solubilizing ability

The isolates were tested for their quantitative ability in solubilizing phosphate by using Pikovskaya broth medium with $Ca_3(PO_4)_2$ as the phosphate source [14]. Materials of Pikovskaya broth medium are glucose 10 g; $Ca_3(PO_4)_2$ 5 g; $(NH_4)_2 SO_4$ 0,5 g; $MgSO.2H_2O$ 0,1 g;MnSO4 25 mg; $FeSO_4$25 mg; KCl 0,2 g, yeast extract 0,5 g; and agar 15 g, dissolved in sterile water until volume of 1 l. Pipette 30 ml of the suspension and put in the Erlenmeyer, containt Pikovskaya broth medium, and incubated in rotary shaker at 150 rpm for 7 days. Filter the 20 ml culture with filter paper Whatman No. 42. Filtrate was centrifugated at 1000 rpm for 15 minutes, 5.0 ml of supernatan then poured into test tubes, added with 0.5 ml of concentrated reagents P (12 g ammonium molibdat, 0.277 g kaliumantimoltartat) and Reagent dye concentrated (0.53 g ascorbic acid), shaked for a few minutes, and let it standed for 30 minutes. The absorbance of solution was measured with the spectrophotometer at a wavelength of 693 nm. In the same way was done in the Erlenmeyer flask containing Pikovskaya broth medium uninoculated fungi as a control.

Fungal Isolate as Plant Growth Promoting Fungi (PGPF)

The role of endophytic fungi as plant Growth Promoting Fungi was tested against rice plants according to Hermawati (2007) [15]. Rice seeds soaked in 1% NaOCl for 1 minute to remove pathogens and contaminants on the viability of the seed. Seeds soaked in a suspension of

endophytic fungi for 1 day. Rice seed germinated in a petri dish that has been coated with 2 sheets of filter paper and moistened. Control were made by soaking the seeds in sterile water. The parameters measured were the percentage of germination and abnormal growth in rice seedlings for 7 days.

RESULTS AND DISCUSSION

Isolation of endophytic fungi from local aromatic rice plants obtained 16 isolates. Ten isolates from stem (KN1, KN2, KN3, KN 4, KN5, KN 6, KN7, KN11, KN 14, KN15), three isolates from leaf (KN9 , KN10, KN13) and three isolates from roots KN8, KN12, KN16). This suggests that the presence of endophytic fungi in plant tissue is not the same and spread randomly. This is consistent with research from Stofwal that the existence of the type and number of endophytic fungi on each part of the plant is not the same [16.17]. Besides being very diverse in nature, these endophytes are a source of novel bioactive secondary metabolites [18.19]

Detection and Quantification of IAA

Endophytic fungi isolates showed the highest IAA production is isolate KN10 (2.651 mgl^{-1}) and lowest KN6 isolates (0.635 mgl^{-1}). Lower than the results of research Nenwani [12], F1 fungal isolates were able to produce IAA 11.45 μgml^{-1} , also production IAA by bacteria in the presence of 5 $mgml^{-1}$ tryptophan is 7.3 to 32.8 $mgml^{-1}$ and 2.68 to 10.8 in the condition without tryptophan $mgml^{-1}$ [20,21]. Similarly, bacteria from maize roots in vitro resulted in the highest IAA on the KB3 isolates 1.1255 ppm [22]. The study of bacterial isolates in the rhizosphere of onion plants produce IAA Sulawesi varies from 0.76 to 2.33 ppm [23]. The highest IAA production produced by *B. subtilis isolates ME 105*, *B. amyloliquefacaciens* subsp. plantarum ME 3, *P. polymyxa*, and *B. amyloliquefaciens* subsp. plantarum ME8 (188, 151.9, 108.1 and 107 μgml^{-1}) [24]

IAA produced by bacteria can promote plant growth by increasing the number of root hairs and lateral roots [25,26]. Endophytic fungi can increase the germination percentage of *Vigna radiata* and *Cicer arietium* by 95 and 87% compared to controls [13]. Gibberellins and auxin may play an important role in plant growth, reproduction, metabolism and response to various environmental cues [27]

Measurement of the IAA concentration produced by each isolate using spectrophotometer showed varying results, namely 0.635 – 2.651 mgl^{-1}. (Table 1).

Table 1. Absorbance and Concentration of IAA from Endophytic Isolates

Isolates	Absorbance (λ)	IAAConcentration (mgl^{-1})
KN1	0.106	1.476
KN2	0.070	0.905
KN3	0.146	2.111
KN4	0.175	2.571
KN5	0.068	0.873
KN6	0.054	0.651
KN7	0.830	1.111
KN8	0.053	0.635
KN9	0.078	1.032
KN10	0.180	2.651
KN11	0.139	2.000
KN12	0.123	1.746
KN13	0.120	1.698
KN14	0.090	1.222
KN15	0.123	1.746
KN16	0.610	1.762

Table 2. Ablity of Endophytic Fingal Isolates in Solubilize Phospate

ISOLATES	ABSORBANCE (λ)	CONCENTRATION (mgl^{-1})
KN1	0.450	2.099
KN2	0.673	3.260
KN3	0.473	2.219
KN4	0.656	3.172
KN5	0.587	2.813
KN6	0.697	3.385
KN7	0.537	2.552
KN8	0.333	1.490
KN9	0.683	3.313
KN10	0.603	2.896
KN11	0.705	3.427
KN12	0.295	1.292
KN13	0.712	3.464
KN14	0.046	0.005
KN15	0.613	2.948
KN16	0.761	3.719

Testing of Isolate as Plant Growth Promoter

Figure 1. Abnormally growth of rice sprouts after inoculation with endophytic fungal isolates

Ability of Endophytic Fungal Isolate in Solubilize Phosfat

Quantitative measurement using spectrofotometer at a wavelength of 693 nm showed that sixteenth endophytic fungal isolates has the ability to dissolve phosphate that varies from 0.005 – 3.719 mgl^{-1} (Table 2). There are 7 isolates showed high phosphate solubility there are KN9, KN13, KN11, KN9, KN6, KN2 and KN2. This is accordance with the results of Pradhan and Sukla, [28,29] that found *Aspergillus sp* and *Penicillium sp* can sequentially dissolving 480 mg ml^{-1} of phosphate from 0.1 tricalsium phosphate (TCP) after 4 days.

The ability of isolates act as PGPF through applications on rice seed showed that of 16 isolates only 1 isolate (KN15) that caused abnormally growth on rice sprouts after 1-7 days. (Figure 1).

CONCLUSION

There are 16 isolates of endophytic fungi from local aromatic rice that has the ability to produce the IAA hormone. The ability of tested isolates in producing IAA, varried from 0.635 to 2.651 mgl^{-1}. Highest IAA production was shown by isolate KN10 (2.651 mgl^{-1}). Similarly, the ability to dissolve phosphate also varied from 0.005 – 3.719 mgl^{-1}. And there is only 1 isolates that showed abnormally growth in rice seedlings.

ACKNOWLEDGEMENTS

Thanks to the Directorate of Higher Education for funding this research through a Doctoral Dissertation Research Grant through Kopertis DIPA IX fiscal year 2014.

REFERENCES

[1] Strobel GA. Natural products from endophytic microorganism. Journal of Natural Products. 2004; 67: 257-268.

[2] Noverita, Dinah Fitria, Ernawati Sinaga.Isolation and antibacterial activity assay of fungal endophyte of leaves and Rhizome *Zingiber ottensii.* (in Indonesia). Jurnal Farmasi Indonesia 2009; 4 : 171 -176

[3] Khan, A. L., M. Hamayun, N. Ahmad, J. Hussain, S. M. Kang, Y.H. Kim,. Salinity stress resistance offered by entophytic fungal interaction between *Penicillium minioluteum* LHL09 and *Glycine max*. L. J. Microbiol. Biotechnol. 2011; 21: 893-902.

[4] Chutima, R. and S. Lumyong. Production of indole-3-acetic acid by Thai native orchid-associated fungi. Symbiosis 2012;56: 35-44.

[5] Radhakrishnan, Ramalingam, kang-bo shim, byeong-won lee, chung-dong Hwang, Suk-Bok Pae, Chang-Hwan Park, Sung-Up Kim, Choon-Ki Lee, and In-Youl Baek. IAA-producing *Penicillium sp.* NICS01 triggers plant growth and suppresses *Fusarium* sp.-induced oxidative stress in sesame (*Sesamum indicum* L.) J. Microbiol. Biotechnol. 2013;23: 856–863

[6] Nakamura A, Umemura I, Gomi K . Production and characterization of auxin-insensitive rice by overexpression of a mutagenized rice IAA protein. Plant J. 2006; 46: 297-306

[7] Bilkay I.S, , Ş Karakoç, N Aksöz, Indole-3-acetic acid and gibberellic acid production in *Aspergillus niger*. Turk J Biol 2010; 34 : 313-318

[8] Oliveira AG, Junior AFC, Santos GR, Miller LO, Chagas LFB. Potencial de solubilização de fosfato e produção de AIA por Trichoderma spp. Rev. Verde. 2012;7:149-155

[9] Resende.M.P, I. C. M C. Jakoby, L C R dos Santos, M. A Soares, F. D Pereira, E.L Souchie, and F G Silva, Phosphate solubilization and phytohormone production By endophytic and rhizosphere Trichoderma Isolates of Guanandi (*Calophyllum brasiliense* Cambess). Afr.J.Microbiol. 2014;27 :2616-2623.

[10] Deshwal, V.K., Devi, M.S., Bhajanka, N., Mistri, J., Bose, A. and Saini, N. *Pseudomonas aeruginosa* strains and their role in plant growth promotion in medicinal plant. global j. appl. agri.res.2011;1: 49-55.

[11] Deshwal V.K., and P Kumar. Plant growth promoting activity of Pseudomonads in rice crop. int.j.curr.microbiol.app.sci.2013;2(11): 152-157

[12] Nenwani.V, P Doshi, T. Saha and S. Rajkumar. Isolation and Characterization of a fungal isolatae for Phosphate solubilization and plant growth promoting. Activity Journal of yeast and fungal research.2011; 1(1) pp. 009-014

[13] Bhagobaty R.K. and S.R. Joshi. Promotion of seed germination of Green gram and Chick pea by *Penicillium verruculosum* RS7PF, a root endophytic fungus of *Potentilla fulgens* L. Advanced Biotech. 2009.

[14] Subba-Rao, S.N.S, 2010. Soil Microorganisms and Plant Growth (in bahasa Indonesia). Penerbit Universitas Indonesia. Jakarta.

[15] Hermawati H. Effect of Endophytic Fungus of the Biology and Population Growth Aphis gossypii Glov (Homoptera : Aphididae) in pepper. (in bahasa Indonesia). Skripsi. IPB. 2007
[16] Stovall, M.E. An investigations of the fungus *Balansia cyperi* and its effect on purple nutsedge, *Cyperus Rotundus*. 1987.
[17] Sunariasih N P L. I Ketut Suada and Ni Wayan Suniti. Identification of Endophytic Fungi from Rice Grain and It's Inhibiting Ability by *In Vitro* Against *Pyricularia oryzae* Cav. E-Jurnal Agroekoteknologi Tropika .2014; 3(2): 51-60
[18]Arnold, A.E.; Lutzoni, F. Diversity and host range of foliar fungal endophytes: Are tropical leaves biodiversity hotspots? Ecology. 2007;88: 541–549.
[19]Sun, X.; Guo, L.D.; Hyde, K.D. Community composition of endophytic fungi in Acer truncatum and their role in decomposition. *Fungal Divers.* 201*; 47: 85–95.*
[20]Ahmad, F., I. Ahmad, and M.S. Khan. 2005. Indole acetic acid production by the indigenous isolates of *Azotobacter* and fluorescent *Pseudomonas* in the presence and absence of tryptophan. Turk J. Biol.2005;29: 29-34.
[21]Widiastuti H, Siswanto, dan Suharyanto. Characterization and Selection of *Azotobacter* sp. In Enhancing Seed Germination and Growth of Plant. Buletin Plasma Nutfah. 2010;16 (2)
[22] Khairani.G. Isolation and Ability Test of Endofphytic Bacteria Producing IAA (indole Acetic Acid) Hormone From Crops Maize Root (*Zea mays L*)(in Indonesia). 2010.
[23] Kafrawi, Baharuddin, E. L.Sengin and A. Rosmana. Screening Of Free-Living Indole Acetic Acid Producing Rhixobaceria From Shallot Rhizosheres In The Island of Sulawesi. International Journal of Scentific and Technoly Research. 2014;3:118-121
[24] El-Meleigi, M. A, Ahmed A. Al-Rogaibah, Gmal H. Ibrahim and Khaled A. Al Gamhan, Role of Antibiosis and production of Indole-3-Acetic acid by bacilli strains in suppression of root pathogens and growth promotion of Alfalfa seedlings Int.J.Curr.Microbiol.App.Sci . 2014; 3 (6):685-696
[25] Okon Y and YKapulnik. Development and function of Azospirillium inoculated Roots. Plant and Soil 1986;90: 3-16
[26] Huen E, Screening of Soil Bacteria for Plant Growth Promotion Activities in vitro. Indonesian Journal of Agricultural Science. 2003; 4 (1): 27-31
[27] Waqas M, A L. Khan, M. Kamran, M.Hamayun, SM Kang, YH Kim and IJ Lee.Endophytic Fungi Produce Gibberellins and Indoleacetic Acid and Promotes Host-Plant Growth during Stress. Molecules. 2012;17: 10754-10773
[28] Pradhan N and Sukla LB. Solubization of inorganic phosphates by fungi isolated from agriculture soil. Arf.J.Biotechnol. 2005;5 (10) : 850 -854
[29] Handayani D. Potency of *Aspergillus* and *Penicillium* from Dipterocarp Leaf Litter as Phosphate Solubilizer and Root Endosymbiont. (in Bahasa Indonesia) Thesis IPB.2011. p.71.

Presented at ISFA (September 16-17, 2014-Semarang, Indonesia) as Paper #33 "Managing Biosafety and Biodivesity of Food from Local to Global Industries"